014277

DATA EVALUATION REPORT

ZIRAM

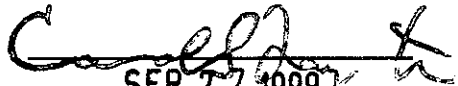
STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY - RAT (83-6)
MRID 43935801 - DP BARCODE D223815

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 99-57

Primary Reviewer: Carol S. Forsyth, Ph.D., D.A.B.T.	Signature: Date:	 SEP 27 1999
Secondary Reviewers: Rosmarie A. Faust, Ph.D.	Signature: Date:	Robert H Ross SEP 27 1999
Robert H. Ross, M.S. Group Leader	Signature: Date:	Robert H. Ross SEP 27 1999
Quality Assurance: Susan Chang, M.S.	Signature: Date:	 SEP 27 1999

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EPA Reviewer: P.S. Gaunt, Ph.D. _____, Date 1/7/00
RRB4 (7509C)
EPA Secondary Reviewer: Sanjivani Diwan _____, Date 1/11/00
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DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity - Rat
OPPTS 870.6300 [§83-6]

DP BARCODE: D223815
P.C. CODE: 034805

SUBMISSION CODE: S501475
TOX. CHEM. NO.: N/A

TEST MATERIAL (PURITY): Ziram (97.8% a.i.)

SYNONYMS: Zinc dimethyldithiocarbamate

CITATION: Nemec, M.D. (1996) A dietary two-generation reproduction and developmental neurotoxicity study of ziram in rats. WIL Research Laboratories, Inc., 1407 George Road, Ashland, OH 44805-9281. Laboratory study number WIL-223003, January 30, 1996. MRID 43935801. Unpublished.

SPONSOR: The Ziram Task Force, NPC, Inc., 22636 Glenn Drive, Suite 304, Sterling, VA 22170

EXECUTIVE SUMMARY: Ziram (97.8% a.i.) was evaluated for developmental neurotoxicity during the conduct of a two-generation reproduction study. Test article was administered to male and female Sprague-Dawley CD rats in the diet at concentrations of 0, 72, 207, or 540 ppm for two generations (MRID 43935801). These concentrations resulted in F_1 maternal doses of 5, 13, and 32 mg/kg/day, respectively, during gestation and 11, 30, and 79 mg/kg/day, respectively, during lactation. The developmental neurotoxicity of ziram was evaluated in the F_2 offspring. Behavioral alterations, motor activity measures, auditory startle response, learning and memory, and the age of sexual maturation (vaginal perforation and balanopreputial separation) were examined. Brain weights and dimensions were recorded, and gross and histopathological evaluation of the nervous system tissue was conducted.

No treatment-related maternal or offspring toxicity was observed in the 72 or 207 ppm groups as compared with controls.

All F_1 dams survived until scheduled sacrifice and there were no treatment-related clinical signs of toxicity or neurobehavioral alterations. The high-dose F_1 females had significantly lower body weights throughout gestation ($p \leq 0.05$ or 0.01) and lactation ($p \leq 0.01$) as compared to controls. Body weight gains were significantly lower in the high-dose ($p \leq 0.01$) group as compared to

controls during days 14-20 of gestation. No significant differences occurred for body weight gains during lactation for any treated group as compared to controls. On gestation day 20 and lactation day 21 body weights of the high-dose F_1 animals were 89% and 93%, respectively of the control level. Food consumption was significantly ($p \leq 0.05$ or $p \leq 0.01$) lower than controls in the high-dose group throughout gestation and lactation. At necropsy, there were no treatment-related gross- or histopathological abnormalities observed in the dams, and differences in absolute and relative organ weights of the high-dose group as compared to controls were consistent with reduced body weights of these animals.

High-dose F_2 pups also had lower body weights than the controls throughout lactation, with significance reached on postnatal days 1, 4 precull (92-93%; $p \leq 0.05$), 14, and 21 (88-91%; $p \leq 0.01$). Mean body weights of the high-dose F_2 males and females were also statistically significantly ($p \leq 0.05$ or 0.01) less than the controls throughout the postweaning period. However, final (postnatal day 70) body weights of F_2 males and females were 93 and 96%, respectively, of the control values. Overall body weight gain of the high-dose males was 94% of the controls while overall weight gain of the high-dose females was 99% of the control value. The age of sexual maturation for F_2 pups was not affected by treatment.

No clinical signs of neurotoxicity were observed in the F_2 offspring during daily cageside observations or at detailed physical examinations. Motor activity (total and/or ambulatory counts) was increased at all treatment levels, often 2 to 3-fold greater than control and in a dose-related manner, in pups of both sexes. At the low dose, these increases are apparent beginning at postnatal day 17 and continuing through postnatal day 21, while at the mid and high doses, they initiate at postnatal day 13 and continue through both days 17 and 21. Motor activity counts for postnatal day 60 were similar for control and treated rats of both sexes. Mean peak startle response was decreased (approximately 30% from control) in an apparently dose and treatment-related manner in high-dose pups of both sexes at postnatal day 22; this finding was not observed on postnatal day 60. Mean latency to peak response, response duration, and average response values appeared to be unaffected in treated animals as compared with controls on postnatal days 22 and 60. Learning and memory evaluations (in a water T-maze) at postnatal days 11 and 70 were similar for control and treated offspring. Brain weights (whole and regional) and dimensions (length and width) were not affected by treatment at postnatal days 11 or 70. Qualitative histopathological evaluation of the nervous system tissues did not reveal any treatment-related findings.

The maternal LOAEL is 540 ppm (32 mg/kg/day) based on reduced body weights and/or body weight gains, and decreased food consumption during gestation and lactation. The maternal NOAEL is 207 ppm (13 mg/kg/day).

The offspring LOAEL is 72 ppm (5 mg/kg/day) based on increased motor activity on postnatal days 17 and 21 for both sexes. The offspring NOAEL is <72 ppm (5 mg/kg/day).

Although this study contains useful information regarding the developmental neurotoxic potential of ziram, it is classified as **Guideline Unacceptable** (§83-6; OPPTS 870.6300) due to the following major deficiencies: 1) Neurobehavioral data (motor activity, startle response, and cognitive function) were not presented as percent change from control or analyzed statistically. 2) Simple morphometric

analysis of representative locations within the neocortex, hippocampus, and cerebellum was not performed for F_2 offspring during histopathological examination of the brain at postnatal days 11 and 70. This study can be upgraded upon the submission and review of acceptable statistical analysis and morphometric data.

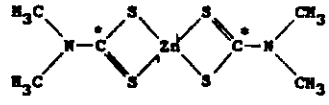
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Ziram

   Description: white powder
   Lot/Batch No.: V528/8331AA
   Purity: 97.8%, a.i.
   Stability of compound: stable
   CAS No.: 137-30-4
   Structure:



2. Vehicle and/or positive control

   Purina® Certified Rodent Chow® #5002, meal form, was used as vehicle and negative control. No positive control was used in this study.

3. Test animals

   Species: rat
   Strain: Sprague-Dawley Crl:CD®BR
   Age and weight of F_0 at start of study: approximately 6 weeks; males: 144-218 g, females: 117-174 g
   Source: Charles River Breeding Laboratories, Inc., Portage, Michigan
   Housing: Parental animals were individually housed in wire mesh cages until mating. Mated females were transferred to plastic maternity cages with nesting material (ground corn cob).
   Diet: Purina® Certified Rodent Chow® #5002, meal form, was available *ad libitum*.
   Water: Tap water was available *ad libitum* via an automatic watering system.
   Environmental conditions:

Temperature: 68 - 79°F
Humidity: 20 - 92%
Air Changes: 10/hour
Photoperiod: 12 hour light/dark
Acclimation period: 12 days

B. STUDY DESIGN

This study was designed to assess the developmental neurotoxicity potential of Ziram when administered in the feed to rats for two generations.

1. In life dates

Start: May 24, 1994; end: March 31, 1995

2. Mating procedure and schedule

F_0 animals were fed control or treated diets for 10 weeks prior to mating and continuing throughout mating, gestation, and lactation. F_1 animals were weaned onto the same diets as were fed their respective parents. After weaning, the F_1 animals were maintained on treatment for 10 weeks prior to mating. For mating, animals of the same dose group were paired one male to one female. Sibling matings were avoided. Females were examined each morning for evidence of mating which consisted of sperm in a vaginal lavage or a copulatory plug. Day 0 of gestation was designated as the day evidence of mating was seen. Each female was left with its first male for a maximum of 10 days. If no sign of mating was observed, the female was placed with a proven male of the same treatment group for an additional 5 days. When evidence of mating was not detected after the total 15-day period, the female was returned to individual housing.

3. Animal assignment

F_0 animals were randomly assigned to groups by the use of a computerized stratification block design based on body weight. F_1 pups were randomly selected, at least one male and one female from each litter, and weaned onto the same diet as their respective parents. Following standardization of litters on postnatal day 4, 30 F_2 pups/sex/group were also randomly selected for pre- and postweaning motor activity, auditory startle response, and learning and memory testing. F_2 pups were not exposed to test article after weaning. Of the 30 selected F_2 pups/sex/group, 16 pups/sex/group were allocated for neuropathology and/or brain weight measurements at termination on postnatal day 70. Animal assignment is given in Table 1.

TABLE 1. F_2 Animal assignment			
Dose Group	**Conc. in Parental Diet[a] (ppm)**	**Male**	**Female**
0 (Control)	0	30	30
1 (Low)	72	30	30
2 (Mid)	207	30	30
3 (High)	540	30	30

Data taken from pp. 28, MRID 43935801.
[a]Diets were administered to the F_0 and F_1 generations only.

4. Validation of testing procedures

Laboratory validation of the testing procedures for developmental neurotoxicity testing were not included in the study report. However, summary reports from positive control and validation studies conducted by WIL Research Laboratories for neurotoxicity parameters were provided in a separate facsimile transmission to the Health Effects Division. These included the following:

A. A validation study of the Digiscan "Micro" Animal Motor Activity System.WIL-99026, 05-NOV-90 thru 30-NOV-90 (positive control materials = d-amphetamine sulfate and chlorpromazine hydrochloride)

B. An acute neurotoxicity study of 1-naphthyl-N-methylcarbamate (carbaryl) in rats. WIL-99032, 03-DEC-90 thru 21-DEC-90

C. Part 1: A repeated dose neurotoxicity study of acrylamide in rats; Part 2: An acute neurotoxicity study of trimethyltin chloride in rats. WIL-99034, 14-JAN-91 thru 19-FEB-91

D. An interobserver reliability study of technicians assigned to perform the F.O.B. obserations for neurotoxicity studies. WIL-99035, 01-AUG-91 thru 09-AUG-91 (positive control material = 3'-3' iminodipropionitrile [IPDN])

These studies appeared to adequately meet the purposes for which they were intended and provided a measure of confidence in the ability of the performing laboratory to conduct and evaluate neurotoxicology studies. However, validation and/or positive control data to support other endpoints applicable specifically to the developmental neurotoxicity study, and to the age of animal evaluated in this study, were not provided. These might include, for instance, auditory startle and habituation, learning and memory testing using the apparatus and test paradigm of choice (the Biel t-maze in this instance),

behavioral testing in immature animals, and postmortem procedures (brain weights and dimensions and neuropathological evaluations) in immature animals.

5. Dose selection rationale

Doses were selected on the basis of a dose range-finding study conducted previously by the testing laboratory (WIL-223002). Only data on the analysis of dietary formulations were included with the main study.

6. Diet preparation and analysis

Fresh diets were prepared weekly and stored refrigerated. All diet formulations were adjusted for the per cent purity of the test article (97.8%). The appropriate amount of test article was added to 5 kg of diet and mixed in a Hobart blender for 5 minutes. This premix was added to a sufficient amount of diet to obtain 17 kg of the appropriate concentration of test diet and blended in a V-twin shell blender for 10 minutes. An intensifier bar was used during the first and last three minutes of this blending time. Samples from each diet were collected weekly and analyzed for concentration. Duplicate samples from the top, middle, and bottom of each dietary mixture were taken prior to initiation of dosing. One set of samples was analyzed for homogeneity while the other was stored refrigerated for 10 days and analyzed for stability.

Results –
Homogeneity analysis: Results from the analyses of samples taken from the top, middle, and bottom of each diet formulation showed the preparations to be adequately mixed. Concentrations varied 97.2-107% of target for all formulations.

Stability analysis: Following 10 days at refrigerated storage, the low-, mid-, and high-dose diets were within 76%, 87%, and 96% of their initial measured concentrations. To compensate for the loss in the low- and mid-dose diets, these formulations were fortified by 20% and 15%, respectively. This ensured that target concentrations were maintained over the course of the study.

Concentration analysis: Absence of test article was confirmed in the control diet. Concentrations of test article in the low-, mid-, and high-dose diets varied 87-101%, 88-103%, and 96-108% of nominal, respectively. If the measured concentration was >15% from target, fresh diets were prepared.

Results of the dietary analyses indicate that mixing was adequate and that administered concentrations were within 13% of target. Loss of test article in the diet due to storage was compensated for by increasing the initial concentrations of the low- and mid-dose diets.

7. Statistical analyses

Statistical tests were two-tailed except where noted. Tests were run on a Digital® MicroVAX® 3400 computer. Numbers of still born and dead pups and pup viability indices were analyzed with a Chi-square test with Yates' correction factor. Pup body weights, mean gestation length, absolute and relative organ weights, and live litter size were analyzed by a one-way analysis of variance (ANOVA) with Dunnett's test. Histopathological findings were analyzed by the Kolmogorov-Smirnov test (one-tailed). Significance level was set at 0.05. Data from motor activity, auditory startle, and Biel maze swimming tests were not subjected to statistical analyses. Time to vaginal perforation or balanopreputial separation was not analyzed statistically.

C. METHODS

1. Parental clinical observations and mortality

All animals were observed twice daily for morbidity, mortality, overt signs of toxicity, and dystocia. Body weights of the F_0 and F_1 males were recorded weekly throughout the study and prior to necropsy. Females were weighed weekly until evidence of copulation, then on days 0, 7, 10, 14, and 20 of gestation, and on days 1, 4, 7, 14, and 21 of lactation. Food consumption for the F_0 and F_1 adults was measured daily until mating. After mating food consumption for males was measured daily until necropsy and for females daily throughout gestation and lactation. Food consumption was calculated and reported as g/animal/day and g/kg/day at weekly intervals. Compound consumption was calculated from the mean food consumption data and the nominal concentration of test article in the diet for each sex/group.

2. Litter clinical observations and mortality

All females were allowed to litter, the pups were examined for gross malformations, and the number of live and dead pups was recorded. Offspring were individually identified by application of tattoo markings on the digits on lactation day 0. Live pups were counted and examined daily for survival and changes in appearance or behavior. Pups were individually sexed on lactation days 0, 4, and 21. Each F_2 pup was weighed and received a detailed physical examination on lactation days 1, 4, 7, 14, and 21, at weekly intervals thereafter until euthanasia, and whenever pups were removed from their cages for neurotoxicity testing. (It does not appear that these examinations were equivalent to the out-of-cage functional observations recommended by guideline for postnatal days 4, 11, 21, 35, 45, and 60.) On lactation day 4, all litters were standardized to 4 males and 4 females, where possible, and the remaining pups euthanized and discarded.

Beginning on postnatal day 40, each F_2 male pup was examined daily for balanopreputial separation. Beginning on postnatal day 30, each F_2 female pup was examined daily for vaginal perforation.

3. Neurobehavioral evaluations

For neurobehavioral evaluations, pups were divided into three replicates to allow for reasonable conduct of the evaluations. Each replicate consisted of pups that were born within 2 days of each other and contained an approximately equal representation of each dose group and sex.

a. Functional observational battery (FOB)

A complete FOB was not conducted on the pups on days 4, 11, 21, 35, 45, and 60 as required by the current guidelines. Clinical observations and physical examinations were done as stated above.

b. Motor activity

Motor activity measurements were made on 10 pups/sex/ group on postnatal days 13, 17, 21, and 60. The motor activity of each animal was monitored for 40 minutes (4, 10 minute subsessions) and divided into total and ambulatory activity based on the number of adjacent photobeam interruptions. A personal computer-controlled system (Digiscan 'Micro' Animal Activity System, Omnitech Electronics, Inc.), consisting of individual test chambers which utilize a series of infrared photobeams surrounding a clear, plastic rectangular cage, was used to quantify each animal's motor activity.

c. Auditory startle response

An auditory startle response test was performed on 10 pups/sex/group on postnatal days 22 and 60 using an automated Auditory Startle Response System. Mean latency to peak, response duration, average response, and peak amplitude were recorded on 5 blocks of 10 trials each.

d. Biel maze swimming trial (learning and memory)

One male and one female from each litter, where available, were assessed for swimming ability and learning and memory using a water-filled, six unit T-maze. The first testing interval was between postnatal days 19 and 23 and the second testing interval between days 58 and 62; the same animals were tested at each interval. Each interval consisted of assessment of swimming ability on day 1 followed by maze learning ability on days 2-5. On days 2 and 3 each animal was tested on the forward direction through the maze and on days 4 and 5 each animal was tested in the reverse direction through the maze. After a three-day rest period, each animal was tested for memory recall of both directions through the maze. The mean number of errors (all four feet into an incorrect channel) and mean escape time for each test day were considered measures of maze-learning ability.

4. Postmortem Studies

a. Sacrifice

All animals were euthanized by carbon dioxide inhalation.

b. Necropsy

1) Parental animals - The F_1 adults were euthanized following weaning of the F_2 pups. A complete necropsy and selective histopathological examination was performed on all animals.

2) Offspring - Offspring dying during lactation days 0 to 4 were necropsied using a fresh dissection technique. Offspring dying on or after day 4 were subjected to a detailed gross necropsy. All F_2 weanlings not selected for developmental neurotoxicity testing were euthanized and necropsied on postnatal day 22. On day 70, all F_2 pups not allocated for neuropathology or brain weight measurements were sacrificed and subjected to gross necropsy. Organ and tissue samples from F_2 pups were collected and saved for histopathological examination as appropriate.

3) Neuropathological evaluations - At postnatal day 11, ten F_2 pups/sex/group were selected for brain weight measurements (whole brain, forebrain, and hindbrain). The brains from 6 of these pups/sex/group were immersion fixed and embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathological examination was performed on tissues from control and high-dose animals. Detailed morphometric evaluation of guideline-specified brain regions (neocortex, hippocampus, and cerebellum) was not conducted on PND 11 F_2 offspring.

On postnatal day 70, 16 animals/sex/group were euthanized and perfused for brain weight measurements and/or neuropathology. Brains from all of these animals were weighed, measured for length and width, and dissected into the forebrain, hindbrain, and cerebellum regions, which were also weighed separately. From 6 of these animals/sex/group, the following central and peripheral nervous tissues (X) were dissected, preserved in paraffin (CNS tissues) or plastic (PNS tissues), sectioned and stained with hematoxylin and eosin. Histopathological evaluation was performed on tissues from males and females in the control and high dose groups. Detailed morphometric evaluation of the neocortex, hippocampus, and cerebellum was not conducted on PND 70 F_2 offspring.

X	brain	X	eyes
	pituitary	X	sciatic nerves
X	spinal cord (C_3-C_8, T_{13}-L_4)	X	tibial nerves
X	lumbar dorsal root ganglia	X	sural nerves
X	lumbar dorsal root fibers	X	peroneal nerves
X	lumbar ventral root fibers	X	cervical dorsal root ganglia
X	trigeminal ganglia	X	cervical dorsal root fibers
X	optic nerve	X	cervical ventral root fibers
			macroscopic lesions

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

All parental animals survived to scheduled necropsy. Clinical signs of toxicity in the treated and control adult animals consisted of hair loss, sores or scabbing around the ears and eyes, and malaligned upper incisors. Although hair loss from the limbs was more common in the male and female high-dose groups than the controls, this trend was not dose-related.

No treatment-related clinical effects were observed in the pups during lactation or postweaning. One F_2 female from the high-dose group was found dead on postnatal day 35; death appeared to be due to a mechanical error resulting in trauma to the head. All other F_2 animals survived until scheduled sacrifice.

B. BODY WEIGHT AND FOOD CONSUMPTION

Body weights, body weight gains, and food consumption during gestation and lactation for the F_1 adult females are given in Table 2. The high-dose group had significantly lower body weights (89-92%) throughout gestation (days 0 and 7, $p \leq 0.05$; day 10, 14, and 20, $p \leq 0.01$) and lactation ($p \leq 0.01$) as compared to controls. Body weight gains were significantly lower in both the mid- ($p \leq 0.05$) and high-dose ($p \leq 0.01$) groups as compared to controls during days 14-20 of gestation. However, overall (GD 0-20) body weight gains of the mid-dose group during gestation were not significantly different from controls. No significant differences occurred for body weight gains during lactation for any treated group as compared to controls. Food consumption was significantly ($p \leq 0.01$) lower than controls by the high-dose group throughout gestation and by the mid-dose group for the day 14-20 interval. During lactation, the high-dose group ate significantly less food than controls throughout (days 1-4 and 4-7, $p \leq 0.05$; days 7-14 and 14-21, $p \leq 0.01$) and the mid-dose group ate significantly less food during the day 7-14 and 14-21 intervals ($p \leq 0.01$).

Body weights of the F_2 pups pre- and post-weaning are given in Table 3. Body weights for males and females during lactation were not separated by the study author. Mean body weights of the low- and mid-dose animals were similar to the controls during both the pre- and post-weaning intervals. High-dose pups had lower body weights than the controls throughout lactation with significance reached on days 1, 4 precull ($p \leq 0.05$), 14, and 21 (p

≤ 0.01). Pup body weights from high-dose litters were 88% of the control value on lactation day 21. Mean body weights of the males and females from high-dose dams were statistically significantly ($p \leq 0.05$ or 0.01) less than the controls throughout the postweaning period. However, after week 38, body weights of the high-dose animals were within 10% of the controls with final body weights of males and females 93 and 96%, respectively of the control value. Overall body weight gain of the high-dose males was 94% of the controls while overall weight gain of the high-dose females was 99% of the control value.

TABLE 2. Maternal body weights, body weight gains, and food consumption values during gestation and lactation

Observation	Treatment Group			
	0 ppm	72 ppm	207 ppm	540 ppm
Mean body weight (g)				
Day 0 of gestation	258 ± 25.0	264 ± 35.3	253 ± 18.1	237 ± 20.3* (92)
Day 20 of gestation	375 ± 31.0	381 ± 42.9	363 ± 25.7	333 ± 28.8** (89)
Day 1 of lactation	289 ± 25.1	298 ± 35.7	285 ± 22.6	263 ± 28.3** (91)
Day 21 of lactation	324 ± 24.0	326 ± 31.2	319 ± 19.4	302 ± 28.2** (93)
Mean body weight gain (g)				
Day 0-20 of gestation	117 ± 17.5	117 ± 17.3	110 ± 14.7	96 ± 12.7** (82)
Day 1-21 of lactation	35 ± 16.3	28 ± 18.3	34 ± 17.3	39 ± 25.0 (111)
Mean food consumption (g/rat/day)				
Day 0-20 of gestation	20 ± 1.8	20 ± 2.6	18 ± 1.7	16 ± 1.7** (80)
Day 1-21 of lactation	48 ± 3.3	47 ± 4.3	44 ± 4.0**	41 ± 4.7** (85)

Data taken from Tables 34-37, 40, and 42, pp. 199-202, 223, and 225, respectively, MRID 43935801.
[a]Numbers in parentheses are percent of control.
Significantly different from control, *p≤0.05; **p≤ 0.01.

TABLE 3: F_2 Body weights during lactation and after weaning (g)				
Study Interval[a]	0 ppm	72 ppm	207 ppm	540 ppm
Preweaning (males and female combined)				
Day 1	6.7 ± 0.61	6.6 ± 0.52	6.6 ± 0.74	6.2 ± 0.48*
Day 4 (precull)	9.6 ± 1.28	9.8 ± 0.90	9.5 ± 1.20	8.8 ± 0.91*
Day 4 (postcull)	9.5 ± 1.32	9.8 ± 0.93	9.5 ± 1.23	8.9 ± 0.94
Day 14	32.9 ± 2.87	33.1 ± 2.60	31.6 ± 3.28	30.0 ± 3.03**
Day 21	46.8 ± 3.94	47.4 ± 3.60	45.1 ± 4.44	41.0 ± 4.68**
Post-weaning - Males				
Week 38	80 ± 8.0	85 ± 5.8	84 ± 10.7	71 ± 11.8** (89)[b]
Week 40	193 ± 16.2	200 ± 13.2	203 ± 16.2	179 ± 18.0** (93)
Week 42	306 ± 23.2	315 ± 25.8	320 ± 19.2	286 ± 23.5** (93)
Week 44	382 ± 28.5	391 ± 35.2	391 ± 24.0	354 ± 21.5** (93)
Weight gain (weeks 38-44)[c]	302	306	307	283 (94)
Post-weaning - Females				
Week 38	75 ± 6.8	77 ± 4.4	75 ± 8.8	66 ± 9.5** (88)
Week 40	145 ± 10.9	147 ± 7.7	147 ± 10.8	134 ± 8.2** (92)
Week 42	187 ± 16.2	188 ± 12.0	189 ± 12.8	178 ± 10.5* (95)
Week 44	223 ± 16.8	221 ± 14.1	227 ± 13.6	213 ± 13.1* (96)
Weight gain (weeks 38-44)[c]	148	144	152	147 (99)

Data taken from Tables 55 and 56, pp. 263 and 264-267, respectively, MRID 43935801.
Significantly different from control, *$p \leq 0.05$, **$p \leq 0.01$.
[a]Study time line: weeks 1-10, F_0 premating; weeks 11-18, F_0 breeding, gestation, and lactation; weeks 19-30, F_1 premating; weeks 31-37, F_1 breeding, gestation, and lactation; weeks 38-44, F_2 post weaning.
[b]Number in parentheses is percent of control.
[c]Calculated by reviewer.

C. MATERNAL TEST SUBSTANCE INTAKE

Based on weekly food consumption and nominal Ziram concentrations in the diet, the doses expressed as mg of test substance/kg body weight/day during gestation and lactation are presented in Table 4. After lactation day 14, it is assumed that the pups were contributing to total food consumption, and thus, were exposed to the test article via both the feed and in the milk. However, the amount of feed consumed by just the dam or by individual pups cannot be determined from the study data.

TABLE 4: Maternal Ziram intake during gestation and lactation (mg/kg/day)

Study Interval	Concentration in Diet		
	72 ppm	207 ppm	540 ppm
Gestation	5 ± 0.5	13 ± 0.9	32 ± 2.2
Lactation			
day 1-4	7 ± 0.9	21 ± 2.8	53 ± 6.0
day 4-7	9 ± 1.0	26 ± 2.5	68 ± 5.4
day 7-14	11 ± 1.0	30 ± 2.5	79 ± 5.9
day 14-21	12 ± 0.9	34 ± 2.8	87 ± 7.4
day 1-21	11 ± 0.8	30 ± 2.4	79 ± 5.3

Data taken from Tables 45 and 46, pp. 237 and 238, respectively, MRID 43935801.

D. DELIVERY AND LITTER DATA

Delivery and litter data are summarized in Table 5. No dose- or treatment-related differences were observed between treated and control groups for duration of gestation, total number of pups delivered, pup survival indices, or per cent male offspring.

There were no differences between treated and control groups in the rate of sexual maturation of the pups as assessed by balanopreputial separation or vaginal perforation. In males from the control, 72, 207, and 540 ppm groups, balanopreputial separation was observed in 100% of the pups by day 50, 46, 46, and 48, respectively. In females, vaginal perforation was observed in 100% of the pups by day 39, 39, 38, and 40, respectively.

TABLE 5: Delivery and litter data				
Observation/study time	**0 ppm**	**72 ppm**	**207 ppm**	**540 ppm**
Females mated	30	30	30	30
Number of litters	23	25	26	28
Mean gestation length (days)	21.8 ± 0.43	21.7 ± 0.45	21.8 ± 0.43	21.7 ± 0.48
Number of live pups	281	306	320	333
Pups/litter	12.2	12.2	12.3	11.9
Number of pups stillborn	4	4	2	3
Sex ratio (% male)[a]	47.0	44.1	53.4	45.3
Survival indices (%)				
Live birth index[a]	98.6	98.7	99.4	99.1
Viability index (precull; d 0-4)	98.9	98.7	98.8	98.5
Viability index (postcull; d 4-21)	89.0	89.9	90.4	90.6

Data taken from Table 47 and 52, pp. 259 and 260, respectively, MRID 43935801.
[a]Calculated by reviewer from group summaries.
Significantly different from control, *p ≤ 0.05, **p ≤ 0.01.

E. NEUROBEHAVIORAL EVALUATIONS

1. Functional observational battery (FOB)

A full FOB was not conducted on the F_2 offspring. No clinical signs indicative of neurotoxicity were observed at either cageside observation or at detailed physical examinations conducted throughout the study.

2. Motor activity

Total and ambulatory activity counts are given in Table 6. Although the study author did not judge the motor activity count data to be indicative of treatment-related toxicity, examination of these summary data reveals apparent dose-related increases in motor activity counts (total and/or ambulatory) in male and female pups at all treatment levels as compared to controls. At the low dose, these increases are apparent beginning at postnatal day 17 and continuing through postnatal day 21, while at the mid and high doses, they initiate at postnatal day 13 and continue through both days 17 and 21. Although there is a high level of variability in the data, mean count values for treated groups were often 2 to 3-fold greater than control, with the greatest differences noted at PND 17 and 21. Preweaning motor activity was observed to generally increase as a function of postnatal age. Motor activity counts for postnatal day 60 were similar for control and treated rats of both sexes. None of these data were statistically analyzed.

The motor activity data were not analyzed or presented in a manner that fascilitated an evaluation of potential differences in habituation between control and treated groups.

TABLE 6: Motor activity counts[a]

Activity	0 ppm	72 ppm	207 ppm	540 ppm
Males				
Day 13				
Total	413 ± 396.7	435 ± 249.9	683 ± 830.3	640 ± 417.9
Ambulatory	185 ± 116.2	248 ± 101.3	312 ± 309.0	343 ± 193.5
Day 17				
Total	581 ± 481.6	903 ± 733.9	1046 ± 1132.5	1203 ± 1018.3
Ambulatory	256 ± 180.0	351 ± 207.0	400 ± 346.9	490 ± 385.0
Day 21				
Total	1242 ± 582.5	2113 ± 897.1	2243 ± 1220.9	2105 ± 607.3
Ambulatory	503 ± 229.2	915 ± 450.3	983 ± 690.4	969 ± 403.4
Day 60				
Total	2318 ± 561.7	1797 ± 744.8	2224 ± 409.3	2214 ± 597.5
Ambulatory	1447 ± 321.0	1173 ± 470.5	1366 ± 252.1	1422 ± 383.5
Females				
Day 13				
Total	312 ± 151.0	399 ± 171.4	474 ± 292.2	466 ± 344.8
Ambulatory	198 ± 116.1	211 ± 77.3	244 ± 103.8	266 ± 159.2
Day 17				
Total	347 ± 220.5	1047 ± 1321.3	1086 ± 779.0	1722 ± 1409.6
Ambulatory	186 ± 103.1	465 ± 634.8	417 ± 218.5	760 ± 624.4
Day 21				
Total	1212 ± 1007.8	1794 ± 1033.8	1690 ± 734.9	2926 ± 1585.8
Ambulatory	518 ± 471.0	861 ± 555.2	703 ± 344.9	1461 ± 860.9
Day 60				
Total	2446 ± 481.2	2537 ± 695.3	2447 ± 636.2	2397 ± 652.0
Ambulatory	1559 ± 232.0	1593 ± 375.0	1557 ± 380.4	1532 ± 467.4

Data taken from Tables 59-62, pp. 271-286, MRID 43935801.
[a]Count defined as a photobeam break; numbers given for total and ambulatory activities are mean total counts for four 10 minute subsessions. N = 10/sex/group

3. Auditory startle response

Startle response data are given in Table 7. Mean peak startle response was decreased approximately 30% from control in high-dose male and female pups at postnatal day 22. In the judgement of Agency reviewers, these decreases appeared to be dose- and treatment-related; the study author did not consider effects on auditory startle to be representative of treatment-related toxicity. Mean latency to peak response, response duration, and average response values appeared to be unaffected in treated animals as

compared with controls on postnatal days 22 and 60. These data were not analyzed statistically.

TABLE 7: Startle response data				
Observation	**0 ppm**	**72 ppm**	**207 ppm**	**540 ppm**
Males				
Day 22				
Peak (g)	92 ± 21.9	89 ± 22.5	83 ± 25.2	69 ± 22.7
Latency to peak (msec)	40 ± 20.0	39 ± 22.3	41 ± 27.2	45 ± 23.2
Response duration (msec)	28 ± 26.7	34 ± 38.6	40 ± 44.0	37 ± 30.4
Average response (g)	50 ± 6.9	52 ± 5.0	51 ± 7.0	42 ± 7.4
Day 60				
Peak (g)	391 ± 70.4	390 ± 65.0	409 ± 65.4	356 ± 43.7
Latency to peak (msec)	48 ± 41.1	50 ± 39.6	54 ± 43.0	49 ± 38.6
Response duration (msec)	113 ± 82.6	129 ± 81.7	140 ± 80.1	136 ± 78.5
Average response (g)	324 ± 25.5	331 ± 27.8	347 ± 13.9	308 ± 5.1
Females				
Day 22				
Peak (g)	78 ± 23.6	78 ± 20.5	73 ± 23.9	56 ± 16.5
Latency to peak (msec)	49 ± 24.3	47 ± 27.8	45 ± 24.5	48 ± 25.4
Response duration (msec)	38 ± 27.9	48 ± 48.0	38 ± 36.8	44 ± 42.1
Average response (g)	43 ± 11.0	49 ± 13.6	42 ± 10.4	33 ± 10.7
Day 60				
Peak (g)	234 ± 41.2	229 ± 14.1	243 ± 21.6	225 ± 37.3
Latency to peak (msec)	58 ± 43.0	60 ± 12.3	54 ± 5.9	54 ± 44.0
Response duration (msec)	118 ± 78.7	144 ± 26.2	119 ± 33.9	119 ± 80.6
Average response (g)	191 ± 19.4	196 ± 6.8	200 ± 17.5	184 ± 11.5

Data taken from Tables 63 and 64, pp. 287-288 and 289-290, respectively, MRID 43935801. Overall mean values for 5 blocks of 10 trials each are presented.

4. Learning and memory

Data from the Biel (T-maze) swimming trials for male and female pups are presented in Tables 8 and 9, respectively. These data were not analyzed statisitically; however, there did not appear to be any adverse effects on swimming ability, learning, and memory function. There were no substantial differences in average times or total errors in the Biel maze swimming trials for any treated group of males or females as compared to their respective controls. For all study groups, mean time to swim the maze in the forward direction decreased from day 2 to 3 of testing, and mean time to swim the maze in the reverse direction decreased from day 4 to 5 of testing. In a similar manner, the mean number of recorded errors decreased over both testing phases. Following a 3-day rest period, the rats of all groups were able to perform the maze task in either direction in a somewhat comparable time frame to that observed in the previous learning trial. At the beginning of the second testing interval (when the rats were 58-62 days of age), mean swimming, learning, and recall times for all study groups were initially lower than or comparable to the shortest times observed in the first testing interval (at 19-23 days of age). It is possible that this enhanced performance may have been related to long-term recall of the task, since the same animals were tested at both intervals.

TABLE 8: Biel swimming trials for male pups				
	0 ppm	72 ppm	207 ppm	540 ppm
Postnatal days 19-23				
Test day 1 - Swimming ability (sec)	12.4 ± 9.43	13.6 ± 14.87	9.1 ± 2.06	17.6 ± 9.21
Test day 2 - Path A				
Time (sec)	81.0 ± 25.11	71.9 ± 29.84	77.1 ± 30.62	71.0 ± 27.65
Errors	15.8 ± 4.50	12.7 ± 4.36	14.6 ± 7.15	13.4 ± 5.09
Test day 3 - Path A				
Time (sec)	42.3 ± 17.42	34.0 ± 15.94	37.6 ± 16.80	33.5 ± 16.52
Errors	8.3 ± 3.68	5.9 ± 4.35	8.5 ± 5.01	6.4 ± 3.64
Test day 4 - Path B				
Time (sec)	73.9 ± 25.48	101.3 ± 43.62	95.6 ± 32.43	77.5 ± 30.96
Errors	13.0 ± 6.25	14.6 ± 5.21	17.7 ± 7.48	11.5 ± 4.72
Test day 5 - Path B				
Time (sec)	48.0 ± 35.15	54.2 ± 30.99	49.1 ± 23.66	48.2 ± 23.52
Errors	9.7 ± 9.72	8.5 ± 5.37	9.4 ± 6.06	8.3 ± 5.61
Test day 6 - Recall				
Time (sec)	59.2 ± 30.87	47.2 ± 16.51	46.0 ± 21.97	60.1 ± 26.58
Errors	13.9 ± 7.62	9.3 ± 3.05	11.1 ± 6.68	13.7 ± 7.03
Postnatal days 58-62				
Test day 1 - Swimming ability (sec)	4.5 ± 0.93	4.8 ± 1.09	4.5 ± 0.82	4.7 ± 0.90
Test day 2 - Path A				
Time (sec)	26.5 ± 8.03	26.7 ± 8.63	26.2 ± 7.65	26.2 ± 4.34
Errors	4.8 ± 2.60	4.2 ± 2.15	5.4 ± 1.70	5.4 ± 2.62
Test day 3 - Path A				
Time (sec)	17.5 ± 7.05	23.0 ± 9.41	14.1 ± 3.83	20.5 ± 7.57
Errors	2.2 ± 1.84	3.4 ± 2.35	1.6 ± 1.52	2.6 ± 1.48
Test day 4 - Path B				
Time (sec)	44.8 ± 20.26	43.9 ± 16.80	38.2 ± 21.65	36.4 ± 7.32
Errors	7.7 ± 4.67	8.4 ± 5.18	6.6 ± 7.04	6.0 ± 2.09
Test day 5 - Path B				
Time (sec)	18.2 ± 5.64	19.5 ± 10.18	18.7 ± 6.07	16.8 ± 4.55
Errors	1.5 ± 1.41	2.3 ± 3.29	2.2 ± 2.49	1.5 ± 1.46
Test day 6 - Recall				
Time (sec)	30.0 ± 14.69	26.2 ± 6.88	23.9 ± 7.81	27.4 ± 8.74
Errors	4.7 ± 4.05	3.5 ± 1.63	3.7 ± 3.32	4.3 ± 2.52

Data taken from Tables 65 and 66, pp. 291-292 and 295-296, respectively, MRID 43935801.
Path A = forward route through maze Time = mean time to escape
Path B = reverse route through maze Error = all four feet into an incorrect channel

TABLE 9: Biel swimming trials for female pups				
	0 ppm	72 ppm	207 ppm	540 ppm
Postnatal days 19-23				
Test day 1 - Swimming ability (sec)	14.2 ± 5.76	10.4 ± 2.88	14.3 ± 8.70	10.5 ± 3.65
Test day 2 - Path A				
Time (sec)	71.6 ± 32.67	80.6 ± 28.07	66.6 ± 27.89	84.2 ± 27.60
Errors	14.1 ± 7.85	16.2 ± 6.30	12.1 ± 5.97	15.6 ± 5.65
Test day 3 - Path A				
Time (sec)	39.5 ± 16.73	32.3 ± 10.66	34.0 ± 14.83	46.3 ± 23.81
Errors	7.7 ± 4.54	6.6 ± 3.24	7.0 ± 4.38	9.2 ± 5.77
Test day 4 - Path B				
Time (sec)	80.0 ± 44.70	88.7 ± 34.94	83.3 ± 28.90	87.2 ± 40.63
Errors	13.5 ± 7.81	15.1 ± 6.78	14.4 ± 6.65	12.5 ± 6.13
Test day 5 - Path B				
Time (sec)	56.8 ± 48.16	43.4 ± 29.42	40.2 ± 14.64	65.0 ± 34.26
Errors	10.1 ± 8.72	6.8 ± 4.94	7.0 ± 3.79	10.8 ± 7.71
Test day 6 - Recall				
Time (sec)	41.6 ± 25.76	42.1 ± 13.58	53.4 ± 23.13	51.4 ± 22.66
Errors	8.3 ± 7.81	9.4 ± 4.55	12.1 ± 3.87	11.9 ± 5.79
Postnatal days 58-62				
Test day 1 - Swimming ability (sec)	4.8 ± 1.41	4.9 ± 1.01	5.4 ± 1.29	5.3 ± 1.16
Test day 2 - Path A				
Time (sec)	34.7 ± 13.78	35.0 ± 18.06	41.3 ± 19.62	32.3 ± 8.09
Errors	5.9 ± 2.87	6.6 ± 3.75	8.1 ± 3.86	6.1 ± 2.35
Test day 3 - Path A				
Time (sec)	25.6 ± 14.20	33.8 ± 13.44	27.6 ± 9.00	32.8 ± 12.50
Errors	3.2 ± 2.84	5.2 ± 2.54	4.3 ± 2.70	5.3 ± 2.13
Test day 4 - Path B				
Time (sec)	42.4 ± 23.19	39.7 ± 9.88	38.2 ± 14.96	45.5 ± 15.16
Errors	6.6 ± 5.25	6.6 ± 2.24	6.3 ± 3.54	7.8 ± 3.59
Test day 5 - Path B				
Time (sec)	18.6 ± 8.54	22.2 ± 7.62	22.2 ± 12.72	26.2 ± 12.26
Errors	1.9 ± 1.80	2.3 ± 2.10	2.6 ± 2.67	3.5 ± 1.91
Test day 6 - Recall				
Time (sec)	34.6 ± 20.29	27.3 ± 11.07	25.6 ± 11.22	34.2 ± 18.72
Errors	5.5 ± 4.38	3.9 ± 2.85	3.5 ± 1.80	5.7 ± 4.86

Data taken from Tables 65 and 66, pp. 293-294 and 297-298, respectively, MRID 43935801.
Path A = forward route through maze Time = mean time to escape
Path B = reverse route through maze Error = all four feet into an incorrect channel

F. NECROPSY RESULTS

1. Gross necropsy

No dose- or treatment-related abnormalities were observed in the F_1 females. No treatment-related gross abnormalities were observed in selected F_2 animals at postnatal days 22 or 70.

2. Organ weights and brain measurements

Selected absolute and relative organ weights at necropsy for the F_1 dams are given in Table 10. Absolute kidney weights were significantly less than controls in the mid- ($p \leq 0.05$) and high-dose ($p \leq 0.01$) groups. Relative brain weights were significantly greater in the high-dose females ($p \leq 0.01$) and relative ovarian weights were significantly greater in the mid-dose females ($p \leq 0.05$) as compared to controls.

Brain weights from day 11 and brain weights and measurements from day 70 are given in Table 11. There were no statistically significant differences between absolute or relative brain weights from treated F_2 pups as compared to controls on postnatal day 11. There were no differences between brain weights, lengths, or widths or in regional brain absolute or relative (to whole brain) weights in treated F_2 groups as compared to controls on day 70. At study termination, high-dose males had slightly greater absolute and relative ($p \leq 0.05$) brain weights as compared to controls. This was not considered to be an adverse effect.

TABLE 10: Selected absolute and relative maternal (F_1) organ weights[a]

Organ	Concentration in the diet			
	0 ppm	72 ppm	207 ppm	540 ppm
Brain				
absolute	1.97 ± 0.091	2.01 ± 0.087	1.96 ± 0.094	1.91 ± 0.094
relative	0.641 ± 0.071	0.671 ± 0.060	0.663 ± 0.059	0.699 ± 0.052**
Kidneys				
absolute	2.36 ± 0.287	2.26 ± 0.238	2.19 ± 0.199*	2.06 ± 0.176**
relative	0.761 ± 0.061	0.747 ± 0.044	0.742 ± 0.074	0.749 ± 0.043
Ovaries				
absolute	0.141 ± 0.020	0.146 ± 0.019	0.149 ± 0.016	0.132 ± 0.022
relative	0.046 ± 0.008	0.048 ± 0.005	0.050 ± 0.007*	0.048 ± 0.008

Data taken from Tables 49 and 50, pp. 245-247 and 248-251, respectively, MRID 43935801.
Significantly different from control, *$p \leq 0.05$; **$p \leq 0.01$.
[a]F_1 animals were necropsied following weaning of the F_2 pups .

TABLE 11: Whole brain weights, lengths, and widths from F_2 rats[a]

Measurement	Concentration in the diet			
	0 ppm	72 ppm	207 ppm	540 ppm
Males				
Terminal body wt day 11 Terminal body wt day 70	24 ± 5.6 384 ± 30.2	26 ± 3.0 401 ± 37.3	24 ± 4.4 385 ± 26.2	23 ± 2.0 357 ± 27.2*
Brain wt day 11 absolute (g) relative (g/100 g)	 1.029 ± 0.155 4.404 ± 0.693	 1.099 ± 0.080 4.215 ± 0.445	 1.042 ± 0.081 4.554 ± 0.809	 1.052 ± 0.076 4.601 ± 0.147
Brain wt day 70 absolute (g) relative (g/100 g)	 2.039 ± 0.083 0.533 ± 0.035	 2.035 ± 0.060 0.511 ± 0.046	 2.045 ± 0.096 0.532 ± 0.029	 2.041 ± 0.083 0.575 ± 0.049*
Brain length day 70 (mm)	20.3 ± 0.68	20.6 ± 0.87	20.6 ± 0.65	20.3 ± 0.41
Brain width day 70 (mm)	15.1 ± 0.63	15.3 ± 0.46	15.0 ± 0.45	14.7 ± 0.63
Females				
Terminal body wt day 11 Terminal body wt day 70	24 ± 1.9 224 ± 13.1	23 ± 2.1 218 ± 17.8	23 ± 2.9 226 ± 11.0	22 ± 3.7 217 ± 14.1
Brain wt day 11 absolute (g) relative (g/100 g)	 1.056 ± 0.066 4.409 ± 0.222	 1.052 ± 0.062 4.510 ± 0.258	 1.043 ± 0.090 4.508 ± 0.369	 0.989 ± 0.075 4.537 ± 0.447
Brain wt at day 70 absolute (g) relative (g/100 g)	 1.871 ± 0.086 0.839 ± 0.049	 1.886 ± 0.091 0.869 ± 0.071	 1.857 ± 0.086 0.824 ± 0.051	 1.853 ± 0.090 0.856 ± 0.068
Brain length day 70 (mm)	18.8 ± 1.65	19.8 ± 0.53	19.4 ± 0.75	19.9 ± 0.41
Brain width day 70 (mm)	14.5 ± 0.54	15.0 ± 0.41	14.4 ± 0.39	14.7 ± 0.39

Data taken from Tables 69, 70, 72 and 73, pp. 303-304, 305-306, 309-312 and 313-314, respectively, MRID 43935801.
Significantly different from control, *$p \leq 0.05$.
[a]N = 10/sex/dose on day 11; 16/sex/dose for weights on day 70; 6/sex/dose for lengths and widths on day 70

3. Histopathological examination

No treatment-related microscopic abnormalities were seen upin qualitative examination of the brains from control or high-dose F_2 animals on days 11 or 70. On day 70, digestion chambers were observed in one control male (lumbar ventral root fibers), one control female (peroneal nerve), and two high-dose males (both sciatic nerve). Swollen axons were also seen in two control males (one of whom also had digestion chambers) in the lumbar ventral and dorsal root fibers, respectively. The study author stated that spontaneous nerve fiber degeneration characterized by digestion chambers has been previously noted in the central and peripheral nervous systems of control and treated rats and should, therefore, not be considered uncommon. Historical control data from the performing laboroatory were not provided to support this position.

No treatment-related histopathological abnormalities were observed for either sex of the F_1 parental generation in the two-generation reproduction study with which this developmental neurotoxicity assessment was combined.

III. DISCUSSION

A. DISCUSSION

Groups of male and female rats were fed Ziram in the diet at concentrations of 0, 72, 207, or 540 ppm for two generations. These concentrations resulted in F_1 maternal doses of 5, 13, and 32 mg/kg/day, respectively, during gestation and 11, 30, and 79 mg/kg/day, respectively, during lactation. The developmental neurotoxicity of Ziram was evaluated in the F_2 offspring. There were no treatment-related clinical signs of toxicity in the parental animals. Reduced body weight gains resulting in lower absolute body weights of high-dose F_1 dams during gestation and/or lactation occurred concurrently with decreased food consumption. Similar results were obtained in other phases of this combination two-generation reproduction study. In the F_0 generation, reduced body weights directly correlated with lower food consumption, especially during the initial few weeks of the study; therefore, the reduced food consumption in early stages of the study was attributed to problems with palatability. However, there is no definative evidence to support the premise that the food consumption deficits observed during gestation and lactation were attributable to reduced palatability, rather than treatment-induced toxicity.

Changes in absolute and relative organ weights of the high-dose animals did not correlate with gross or histological findings and are probably due to the lower body weights instead of a direct effect by the test article. No dose- or treatment-related differences were observed between treated and control groups for duration of gestation, total number of pups delivered, pup survival indices, or percent male offspring.

No treatment-related clinical signs of toxicity were observed in the pups during lactation or after weaning. The death of one high-dose F_2 female pup on day 35 appeared to be accidental and unrelated to treatment. Pups from high-dose dams had significantly lower body weights throughout lactation and continuing postweaning until study termination. Although postweaning body weight gains in the high dose males and females were similar to the control levels, absolute body weights remained lower.

No clinical signs of neurotoxicity were observed in the F_2 offspring during daily cageside observations or at detailed physical examinations.

Although the study author did not consider the motor activity data to demonstrate evidence of treatment-related toxicity, Agency reviewers determined that mean motor activity counts (total and/or ambulatory) were increased at all treatment levels, often 2 to 3-fold greater than control and in a dose-related manner, in male and female pups. At the low dose, these increases are apparent beginning at postnatal day 17 and continuing through postnatal day 21, while at the mid and high doses, they initiate at postnatal day 13 and continue through

both days 17 and 21. The greatest differences are noted at PND 17 and 21. However, motor activity counts for postnatal day 60 appeared to be generally similar for control and treated rats of both sexes. This pattern of response could be related to the fact that the pups would have been potentially receiving ziram in the maternal milk as well as in treated feed, perhaps at levels that were higher than those of the dams. For that reason, effects on the motor activity of pups at the low and mid-dose levels (which were not shown to be maternally toxic) are not considered to be unequivocal evidence of enhanced sensitivity of the pups to ziram exposure.

Mean peak startle response was decreased (approximately 30% from control) in an apparently dose and treatment-related manner in high-dose pups of both sexes at postnatal day 22. However, this finding was not observed on postnatal day 60, again suggesting that this response may have been related to increased test substance intake by pups, via the maternal milk and the diet, in the late lactation period. Mean latency to peak response, response duration, and average response values appeared to be unaffected in treated animals as compared with controls on postnatal days 22 and 60. The study author did not consider that the auditory startle data demonstrated a treatment-related effect.

Learning and memory evaluations (in a water T-maze) at postnatal days 11 and 70 were similar for control and treated offspring.

Brain weights (whole and regional) and dimensions (length and width) were not affected by treatment at postnatal days 11 or 70. Significantly increased relative brain weights of high-dose males on postnatal day 70 were attributed to the lower final body weights of these animals. Qualitative histopathological evaluation of the nervous system tissues did not reveal any treatment-related findings, although positive control data were not submitted by the registrant to support claims made in the study report. More significantly, morphometric evaluations of the neocortex, hippocampus, and cerebellum were not conducted on PND 11 and 70 brains.

Although not required by guideline, it was noted that cholinesterase activity in the brain and/or blood was not measured for either dams or pups in this study. In a dietary subchronic neurotoxicity study with ziram (MRID 43413701), brain cholinesterase inhibition was demonstrated in adult female rats at treatment levels of 207 ppm (16 mg/kg/day) and above, with an NOAEL established for this effect at 72 ppm (6 mg/kg/day).

The maternal LOAEL is 540 ppm (32 mg/kg/day) based on reduced body weights and/or body weight gains, and decreased food consumption during gestation and lactation. The maternal NOAEL is 207 ppm (13 mg/kg/day).

The offspring LOAEL is 72 ppm (5 mg/kg/day) based on increased motor activity on postnatal days 17 and 21 for both sexes. The offspring NOAEL is <72 ppm (5 mg/kg/day).

B. STUDY DEFICIENCIES

A functional observation battery (FOB) was not conducted on the F_2 animals. Since detailed physical examinations were conducted at regular intervals during the study, the absence of FOB data was not considered to compromise the study.

Data from developmental landmarks and behavioral testing (motor activity, auditory startle, and learning and memory) were not analyzed statistically by the study authors. Since apparent treatment-related effects were noted in the motor activity and auditory startle data, these analyses are critical to the interpretation of the study findings. The motor activity and auditory startle data should also be presented as percent chenge from control values.

Morphometric evaluations of the neocortex, hippocampus, and cerebellum were not conducted on the F_2 animals at either the PND 11 or 70 histopathological examination of the brain. These data are required.

Because this study was part of a 2-generation reproduction study, F_2 animals (used for developmental neurotoxicity testing) were identified by the F_0 dam number making it extremely difficult to tell which F_2 pups were from which F_1 dam. Only by comparing both males and females from a litter could the F_1 dam be identified.

Body weight data for F_2 animals was reported as week of study while all neurobehavioral evaluations were conducted and reported as postnatal day of age. This made it impossible to compare the two parameters at concurrent time points.

No historical control data were submitted to support the interpretation of qualitative histopathological findings.

Positive control and validation studies in adult rats that were submitted to the Agency appeared, case-by-case, to adequately meet the purposes for which they were intended. However, validation and/or positive control data to support other endpoints applicable specifically to the developmental neurotoxicity study, and to the age of animals evaluated in this study, were not provided. These are described more extensively in the text above.

C. CORE CLASSIFICATION

Although this study contains useful information regarding the developmental neurotoxic potential of ziram, it is classified as **Guideline Unacceptable** (§83-6; OPPTS 870.6300) due to the following major deficiencies: 1) Neurobehavioral data (motor activity, startle response, and cognitive function) were not presented as percent change from control or analyzed statistically. 2) Simple morphometric analysis of representative locations within the neocortex, hippocampus, and cerebellum was not performed for F_2 offspring during histopathological examination of the brain at postnatal days 11 and 70. This study can be upgraded upon the submission and review of acceptable statistical analysis and morphometric data.